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EVENT ABSTRACT

Anionic surfaces with minimal charge density can restore burst coagulation of microparticle/exosome-depleted blood plasma

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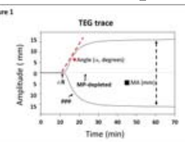
Introduction: Blood contains microparticles (MP) and exosomes derived from a variety of cell types including activated platelets. MPs are procoagulant because their surfaces can express tissue factor and/or calcium-binding sites for coagulation factors that cooperate to induce burst thrombin activation [1]. Platelet-poor plasma (PPP) contains low levels of MPs and can be induced to coagulate after contact with both hydrophilic and hydrophobic surfaces [2]. In a standard thromboelastography (TEG) assay with plastic cups and pins, PPP undergoes spontaneous burst coagulation, as revealed in the TEG trace by a variable clotting time (10 to 26 minutes), a low Angle (~33°) and reproducible low clot tensile strength or Maximum Amplitude (MA ~28 mm) [3] (Fig. 1). PPP coagulates faster and more reproducibly in TEG cups and pins coated with anionic carboxylate-rich nanolayers suggesting FXII activation [2], however the role of MPs is unclear. This study tested the hypothesis that MPs are necessary for PPP burst coagulation in plastic TEG cups, and that anionic surfaces are sufficient to restore burst coagulation of MP-depleted plasma.

Figure 1

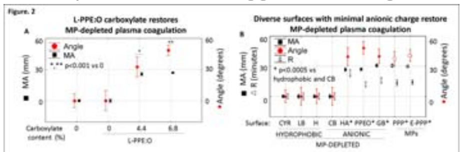


The symmetric TEG trace arises when the cup and pin are connected by fibrin polymerization, allowing measures of clotting time (R, minutes), the burst kinetics of fibrin polymerization (Angle, degrees), and clot tensile strength (Maximum Amplitude, MA, mm). Representative TEG curves are shown for PPP or MP-depleted plasma coagulated in Cyrolite® plastic TEG cups and pins.

Materials and Methods: Plastic Cyrolite® TEG cups and pins (Hemoscope, IN, USA) were modified by plasma-enhanced chemical vapor deposition to make hydrophobic (PP-HMDSO, poly(hexamethyldisiloxane) or variable anionic (L-PPE:O, 0, 4.4% or 6.8% carboxylate functional group content) nanocoatings [3]. Coagulation in the TEG cup was initiated by combining i) human citrated PPP (Precision Biologics, NS, Canada), ii) MP-depleted plasma (by ultracentrifugation 150,000xg, 30 min) or iii) PPP 2xenriched for MP, with 20 µL of 200 mM $CaCl_2$ containing 2.5 mg/mL 10 µm diameter beads (latex, borosilicate glass, carboxylated plastic), or 0.25 to 25 mg/mL hydroxyapatite (HA) nanoparticles (2.03 Ca/P ratio) [4]. Bead composition was analyzed by XPS. Statistical tests used ANOVA (Statistica).

Results: Glass beads with 9% Si and 4% Ca content triggered the fastest PPP clotting time (p<0.05 vs all hydrophobic surfaces). PPP burst coagulation was enhanced by glass beads, L-PPE:O (6.8% carboxylate), and HA nanoparticles, as shown by the Angle increasing from 36° to >55°. HA nanoparticles accelerated PPP burst coagulation in a dose-dependent manner (p=0.004). MP-depleted plasma failed to clot in plastic TEG cups (Fig. 1), and burst coagulation was progressively restored by L-PPE:O with 4.4 to 6.8% carboxylate content (p<0.001, Fig. 2A). MP-depleted plasma coagulation was also induced by 2.5 mg/mL glass beads and 25 mg/mL HA nanoparticles but not by Latex beads, HMDSO, or 1% carboxylated beads (Fig. 2B). In MP-depleted plasma, L-PPE:O with 6.8% carboxylate content triggered the highest Angle (50°) with a clot tensile strength similar to PPP and MP-enriched PPP (Fig.2B).

Figure. 2



TEG data showing (A) gradual restoration of coagulation in MP-depleted plasma with increasing L-PPE:O carboxylate content and (B) restored burst coagulation of MP-depleted plasma by strongly anionic (HA nanoparticles, PPEO: 6.8% carboxylated L-PPE:O, GB: glass beads) but not by hydrophobic (CYR: Cyrolite® TEG cup, LB: latex beads, H: HMDSO) or weakly anionic (CB: 1% carboxylated beads) surfaces. PPP: platelet-poor plasma. E-PPP: PPP enriched 2x for MPs. Mean ± 95% confidence intervals.

Discussion: Glass beads, >4.4% carboxylated surfaces and HA were effective MP biomimetics that restored burst coagulation. These results suggest that inorganic anionic surfaces can serve as functional binding sites for the calcium-binding gla domain of factors

FIXa, FXa and prothrombin. This study is consistent with the notion that tenase and prothrombinase form side-by-side on the same anionic surface [1].

Conclusions: A minimal 6.8% anionic charge group density is required to bring tenase and prothrombinase in close physical proximity for optimal cooperative behavior.

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References:

[1] Owens, A.P., III and N. Mackman, Microparticles in Hemostasis and Thrombosis. Circulation Research, 2011;108(10): 1284-1297.

[2] Zhuo, R., C.A. Siedlecki, and E.A. Vogler, Autoactivation of Blood Factor XII at Hydrophilic and Hydrophobic Surfaces. Biomaterials, 2006;27(24):4325-4332.

[3] Contreras-García, A., Y. Merhi, J.-C. Ruiz, M.R. Wertheimer, C.D. Hoemann, Thromboelastography (TEG) cups and Pins with Different PECVD Coatings: Effect on the Coagulation Cascade in Platelet-poor Blood Plasma. Plasma Processes and Polymers, 2013;10(9):817-828.

[4] D'Elia, N.L., N. Gravina, J.M. Ruso, J.A. Laiuppa, G.E. Santillan, P.V. Messina, Manipulating the bioactivity of hydroxyapatite nano-rods structured networks: Effects on mineral coating morphology and growth kinetic. Biochimica Biophysica Acta-General Subjects, 2013; 1830(11):5014-5026.

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